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Effect of amino acid sequence on the hydrophobicity of small peptides

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Abstract

Hydrophobic adsorbents, which contain alkyl ligands of various chain lengths, were used to study the hydrophobic interactions between alkyl chains and small peptides. We investigated the adsorption of four similar peptides: GWWG, GWGW, WGWG, WGGW. All of them contained two glycines (G) and two tryptophans (W) but the amino acids were arranged in different orders. The capacity factors of peptides between 10 and 35 °C were measured and then the thermodynamic parameters, such as enthalpy and entropy changes (ΔH° and ΔS°) of adsorption, were estimated.

It implied that enthalpy was the major driving force in all the adsorption processes. Furthermore, ΔH° and ΔS° became more negative as the alkyl chain length was increased. It revealed that the van der Waal's interaction had greater influence on the adsorption as the chain length increased. It was also found that, the contribution of each amino acid to peptide's hydrophobicity was affected by the position of the amino acid. When a hydrophobic amino acid was positioned in the middle of a peptide chain, it exhibited the highest hydrophobicity. Interestingly, tryptophan at the carboxyl end was found more hydrophobic than it at the amino end of the peptide.

Keywords: Hydrophobic interaction; Gibbs free energy; Enthalpy; Entropy; Hydrophobic amino acids; Octyl; Decyl-CM-sepharose

1. Introduction

Hydrophobic interaction played an important role in various biological systems and biochemical related applications. For example, the interaction between cells and artificial surface is strongly related to the adsorbed proteins on the surface [1–3]. The adsorption of proteins from liquid medium is often driven by the so-called hydrophobic interaction [4,5]. It was also a dominant interaction between the antigen–antibody binding [6,7] and the association between apolar hormones and their corresponding cell surface receptors [8]. Furthermore, the hydrophobic interaction chromatography is a direct application to use this type of interaction to separate biomolecules [9–13]. The study on how various peptides and proteins interact with hydrophobic surfaces has long been a major research focus [5,14,15].

Hydrophobic interaction is contributed not by a single force but by the joined efforts of various inter-molecular forces, such as van der Waal's forces, electrostatic interactions and hydrogen bonds. Unlike electrostatic interaction that has been well characterized by Poisson equation, hydrophobic interaction can only be characterized experimentally because of its complexity. To find a way to quantify the hydrophobic interaction is an important matter.

Hydrophobic interaction chromatography (HIC) has recently been adopted to study the hydrophobic interaction of proteins. Thermodynamics properties were used to characterize the retention behaviors. Haidacher et al. [16] investigated the effects of temperature on the retention of dansyl amino acids. They found the adsorption of dansyl amino acids on HIC columns (Spherogel, Synchropak propyl and butyl-NPR) was entropy-driven at low temperatures and

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enthalpy-driven at high temperatures. Furthermore, Vailaya and Horvath [17] studied the effect of molecular structure and types of stationary phase on the adsorption enthalpy and entropy. They found that both the adsorption enthalpy and entropy of dansyl amino acids on HIC columns became more positive as the size of amino acids.

The hydrophobicity of amino acids and proteins can be scaled by their retention factors on the column [18]. However, the hydrophobicity of a protein is not only affected by its amino acid composition but also related to the size, the secondary structure and the 3D structure of the protein. Therefore, the hydrophobicity of each amino acid has no direct correlation to the hydrophobicity to a protein, since the arrangement of amino acid in a peptide may also affect the overall hydrophobicity. Understanding the sequence-dependent hydrophobic behavior for small molecules can be helpful for estimating the affect of sequence in large proteins. We attempt to estimate the hydrophobicity of small peptides of the same amino acid composition but in different sequences. Four peptides GWWG, GWGW, WGWG and WGGW were under investigation. The capacity factors of these peptides on various hydrophobic interaction columns are measured between 10 and 35 °C. The Gibbs energy, enthalpy, and entropy of adsorption are calculated. The effect of amino acid sequence and the effect of solid surface are discussed.

2. Experimental

2.1. Material

Tetrahydrofuran (THF), pyridine, hydrochloride and sodium chloride were purchased from Riedel-deHaën (Chinosol, Seelze, Germany). Ammonium sulfate and tris(hydroxymethyl) aminomethane were purchased from Merck (Germany). Butyl-, hexyl-, octyl-, decylamine, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodode (EDC) were obtained from Aldrich (Milwaukee, WI, USA), *N*-hydroxysuccinimide (NHS), glycyl–glycyl–glycyl–glycine (GGGG), were purchased from Sigma (St. Louis, U.S.A). CM-Sepharose gel was purchased from Pharmacia Biotech. (Uppsala, Sweden). The peptides, glycyl–tryptophanyl–tryptophanyl–glycine (GWWG), tryptophanyl–glycyl–tryptophanyl–glycine (WGWG), glycyl–tryptophanyl–glycyl–tryptophan (GWGW) and tryptophanyl–glycyl–glycyl–tryptophanyl (WGGW) were obtained from Digital GENE Biosciences (Taipei, Taiwan).

2.2. Synthesis of the hydrophobic adsorbents

The HIC adsorbent was synthesized by coupling alkylamine to CM-Sepharose particles through the carbodiimide mediated coupling reaction. Firstly CM-Sepharose was washed extensively with deionized water to remove ethanol. The resins were then washed with THF before the addition of alkylamine, EDC and NHS. The mixed was reacted for 20 h at 25 °C in a shaker incubator. After reaction, the CM-Sepharose gel was washed continuously by 20% ethanol solution containing 0.2 M NaCl until no alkylamine can be detected. The eluted alkylamine was tested by ninhydrin reaction. The resulting alkyl-CM-Sepharose may be subject to repeated reaction to obtain higher alkyl substitution. The alkyl content was estimated by titrating the remaining carboxyl groups.

2.3. Chromatographic operation

Hydrophobic interaction chromatography was carried out through a HPLC system which include a GL Sciences model PU-610 pump (Tokyo, Japan), a Lab Alliance model 500 UV monitor (PA, U.S.A.), a Spark model 816 autosampler (Emmen, Holland). The signals were processed through a Chem-Lab data station (SISC, Taipei, Taiwan). The volume of the sample loop is 100 μL. Five milliliters of hydrophobic beads were packed in a 10 cm × 1 cm Sigma jacketed column at a flow rate of 1 mL/min. The final bed height was kept at 5.5 cm. The column temperature was controlled by Firstek B402-D (Shinjuang, Taiwan) circulation water bath. The column was first equilibrated with 20 mM Tris buffer (pH 6) containing $(NH_4)_2SO_4$ (0.25 M) and all the samples were eluted isocratically in the same elution buffer. Eluent was monitored at UV 220 nm through a Lab Alliance UV detector (model 500).

2.4. Calculation of the thermodynamics parameters

We may assume that the adsorption reaction takes place as follows.

$$M + L \rightarrow ML \tag{1}$$

where M represents the adsorbed molecule and L represents the ligand on the adsorbent matrix. Then, the equilibrium constant could be calculated as follows.

$$K_{eq} = \frac{C_{ML}}{C_M C_L} \tag{2}$$

where C_M, C_L and C_{ML} are the concentrations of M, L and ML in the liquid phase.

$$K_{eq} = \frac{n_s}{n_m}\frac{1}{C_L} = \frac{k'}{C_L} \tag{3}$$

where n_s and n_m represent the amount of solute in the stationary phase and mobile phase, respectively. The retention factor (k') can be measured by chromatography and can be related to the equibrium constant (K_{eq}) as:

$$k' \equiv \frac{t_R - t_0}{t_0} = \frac{C_{ML}}{C_M} = K_{eq}C_L$$

where t_R is the retention time of the sample and t_0 is the retention time of non-retained molecules (GGGG). Therefore,

$$k' = K_{eq}C_L \approx K_{eq}L_m \tag{4}$$